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Wildfire effects on soil bacterial community and its potential functions in a permafrost region of Canada

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ABSTRACT

Boreal forests in permafrost zone store significant quantities of carbon that are readily threatened by increases in fire frequency and temperature due to climate change. Soil carbon is primarily released by microbial decomposition that is sensitive to environmental conditions. Under increasing disturbances of wildfire, there is a pressing need to understand interactions between wildfires and microbial communities, thereby to predict soil carbon dynamics. Using Illumina MiSeq sequencing of bacterial 16S rDNA and GeoChip 5.0K, we compared bacterial communities and their potential functions at surface and near-surface permafrost layers across a chronosequence (> 100 years) of burned forests in a continuous permafrost zone. Postfire soils in the Yukon and the Northwest Territories, Canada, showed a marked increase in active layer thickness. Our results showed that soil bacterial community compositions and potential functions altered in 3-year postfire forest ($Fire_3$) comparing to the unburned forests. The relative abundance of Ktedonobacteria (Chloroflexi) was higher in $Fire_3$ surface soils, while Alphaproteobacteria and Betaproteobacteria (Proteobacteria) were more abundant in unburned ones. Approximately 37% of the variation in community composition can be explained by abiotic variables, whereas only 2% by biotic variables. Potential functional genes, particularly for carbon degradation and anammox, appeared more frequent in $Fire_3$ than in unburned soils. Variations in functional gene pools were mainly driven by environmental factors (39%) and bacterial communities (20%; at phylum level). Unexpectedly, wildfire solely altered bacterial communities and their functional potentials of the surface layers, not the near-permafrost layers. Overall, the response of bacterial community compositions and functions to wildfire and the environment provides insights to re-evaluate the role of bacteria in decomposition.

1. Introduction

The boreal forest accounts for 24% of the land in the northern hemisphere (Zhang et al., 2003), and 80% of it is underlain by permafrost (Helbig et al., 2016). Wildfire in the boreal forest appears more frequent due to climate change (Bergeron and Flannigan, 1995; McGee et al., 2014; Wang et al., 2015) and in turn intensifies climate change itself. In permafrost regions, apart from direct CO_2 emissions from biomass burning, the accumulation of charcoal subsequently thaws permafrost by directly absorbing solar radiation (Michaelides et al., 2019). The soil layer above permafrost is known as the active layer, which thaws and freezes seasonally, and its thickness is often determined by temperature between summer and winter (Kane et al., 1991; Zhang, 2005). In recent decades, the active layer thickness has increased in many regions because of global warming (Overland et al., 2014). Permafrost stores roughly 50% of the estimated belowground organic carbon (C) globally (Tamocai et al., 2009). With such significant quantities of C, permafrost thaw exposing organic C to microorganisms (Pautler et al., 2010) becomes particularly important to assess C cycle and climate change.

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Wildfires have large impact on environment conditions that can affect soil C release. For instance, vegetation removal reduces litter production, and thereby limits the supplies of C and nutrients to microorganisms (Jobbagy and Jackson, 2000). In addition, a significant impact of wildfire on soil is not only a loss of soil organic matter (SOM), but a decrease in SOM bioavailability (Aaltonen et al., 2019; Almendros and Gonzalez-Vila, 2012). Wildfires also lead to various changes in ecosystem functions, such as the soil microclimate and nutrient dynamics (Certini, 2005; Galang et al., 2010; González-Pérez et al., 2004). For example, wildfires cause soil nutrient depletion through leaching and erosion (Almendros and Gonzalez-Vila, 2012; Vega et al., 2013), and typically result in an increase in soil temperature and a deeper active layer (Michaelides et al., 2019; Rocha et al., 2012). Because of these complex changes postfire, whether SOM decomposition increases or decreases with wildfire remains uncertain, although rising temperature postfire often increases C turnover rates in boreal forest soils (Bergner et al., 2004; O'Neill et al., 2003).

Environmental factors play critical roles in soil microbial communities, leading to the question of whether and how microbial communities alter with fire disturbances and thereby affect soil C release. Although many studies have investigated the effects of wildfires on bacterial communities in soil ecosystems (Kennedy and Egger, 2010; Smith et al., 2008; Yeager et al., 2005), fire effects on permafrost are only detected recently (Taş et al., 2014). Given the importance of permafrost thaw on global C cycles, it is obliged to understand whether and how wildfires affect microbial activities in permafrost regions. Soil bacteria are presumably the most abundant and diverse organisms on earth (Delgado-Baquerizo et al., 2018). Bacterial communities and their growth rate are primarily determined by soil temperature (Díaz-Raviña et al., 1994; Pietikäinen et al., 2005; Rinnan et al., 2009), moisture (Iovieno and Bååth, 2008), pH (Bååth et al., 1995; Rousk et al., 2011) and substrate availability (Meidute et al., 2008; Rousk and Bååth, 2007). In boreal forest soils, Actinobacteria, Acidobacteria and Proteobacteria are typically dominant (Steven et al., 2007; Sun et al., 2016; Taş et al., 2014; Wilhelm et al., 2011). Members of Proteobacteria such as Betaproteobacteria and Gammaproteobacteria appear more abundant in the rhizospheres of boreal forest plants than in the surrounding soils (Timonen et al., 2017). Some phyla, such as Firmicutes, can survive at high temperature by producing spores (Smith et al., 2008). Moreover, the charcoal amendment following a fire also appears to alter the abundance of nutrient cycling bacteria (Ball et al., 2010).

Researchers also observed that the diversity of bacteria in permafrost soils is higher than that of fungi and archaea (Jansson and Taş, 2014; Steven et al., 2007). Thus, bacteria play an important role in SOM decomposition in permafrost. However, many genes in soil bacteria share functional redundancy (Burke et al., 2011; Louca et al., 2017), rendering the prediction of changes in microbial-driven processes in response to disturbance difficult (Fernández et al., 1999, 2019; Sun et al., 2016). Therefore, to better explore bacterial decomposition process, it is necessary to link bacterial functionalities to community compositions and the environment.

In boreal forests, wildfire sweeps an average of 1–1.5% of the land area each year (Flannigan et al., 2009), and the fire return interval (i.e. the time between fires in a defined area) typically ranges from 50 to > 100 years in a Canadian boreal forest (Coops et al., 2018; De Groot et al., 2013). After a fire, permafrost recovers as a result of forest regeneration and an accumulation of soil organic matter (Jafarov et al., 2013; Shur and Jorgenson, 2007). Although debate continues to surround the duration of permafrost recovery from wildfires, a study using a 2000 year-old chronosequence in boreal forests found that fire-affected soil characteristics, such as the organic layer thickness, nutrient contents of leaves and soil temperature, took < 80 years to recover (Ward et al., 2014). Considering the trade-offs between the short fire return interval of the boreal forest and its typical recovery period from fire, forest stands aged over 100 years in this study were used as the control. We selected four forest areas with 3 ($Fire_3$), 25 ($Fire_{25}$), 46 ($Fire_{46}$) and > 100 years (control) since onset of forest recovery in a continuous permafrost region of northern Canada. We hypothesized that soil bacterial communities in burned forests were taxonomically and functionally different from those in unburned forests. We investigated bacterial communities and their functional gene pools using Illumina MiSeq sequencing of bacterial 16S rDNA genes coupled with GeoChip 5.0K analysis to (i) identify which taxa assemblages are characteristic of burned forest soils and how they change with the environment and (ii) determine which and how potential functions of bacterial communities, especially functions related to C dynamics, change following a fire.

2. Material and methods

2.1. Study areas and sampling

Sampling areas were located in a continuous permafrost zone in the Yukon and the Northwest Territories (66°22′ N – 67°26′ N, 136°43′ W – 133°45′ W) (Köster et al., 2017). Soils of the Cryosolic order occur throughout northern Canada (Stanek, 1982). The bedrock in the study areas consists of Cretaceous sandstones covered by ice-rich fluvial and clay-rich colluvial deposits, with continuous permafrost underneath (Hadlari, 2006). The dominant tree species consist of black spruce [*Picea mariana* (Mill.) Britton, Sterns, and Poggenburg], white spruce [*Picea glauca* (Moench) Voss] and the dominant dwarf shrubs including lingonberry (*Vaccinium vitis-idaea* L.), cloudberry (*Rubus chamaemorus* L.), bog bilberry (*Vaccinium uliginosum* L.) and *Rhododendron groenlandicum* Oeder.

Soil sampling was conducted in July 2015 in four types of forests (areas) that burned 3 (fire in 2012, $Fire_3$), 25 (fire in 1990, $Fire_{25}$), 46 (fire in 1969, $Fire_{46}$) and > 100 years ago (control) along the Dempster Highway. In each area we established three sampling lines that were at least 200-m apart to ensure statistical independence. These lines were treated as replicates in the statistical analysis. Each line consisted of three sample plots which were spaced 50-m apart from each other (Köster et al., 2017). Each sample plot was a circular plot of 400 m^2 and was treated as a pseudo replicate in our statistical analysis. The control line of an unburned forest was placed next to each burned forest area (Fig. A.1). Soil samples were taken from each plot at 5- (surface layer), 10- (middle layer) and 30-cm (deep layer) depths that measured from the soil surface excluding the litter layer. In total, 107 samples [4 forest areas × 3 stands × 3 plots × 3 layers, with one sample missing] were available for analysis. All the sampling areas are relatively flat. Postfire ages of the burned areas were determined based on Canadian government GIS data (Geomatics Yukon, 2011), whereas the forest age of the control was determined by taking increment cores from the largest tree in the sampling plot using an increment borer.

The active layer thickness of the control was 29 ± 1.0 cm during the summer, while that of the $Fire_3$ was much deeper (101 ± 9 cm; Table 1). Thus, soil samples at the 30-cm layer in the control were on near-surface permafrost layers, and those in the remaining forest areas were on active layers. The organic layer thickness of the control was 16.0 ± 1.4 cm but declined to 5.3 ± 1.2 cm in $Fire_3$ (Table 1). As a result, soils collected from 10-cm layer in $Fire_3$ were mineral soils, while that from the remaining areas were organic soils (see Table 1). Soils were taken using a cylinder that horizontally inserted into each layer of the soil profile. Samples for DNA extraction were collected from homogenized soils taken from cylinder using a 2-ml Eppendorf vial. Those samples were transported in liquid nitrogen (180 °C) with dry shipper, and stored at −80 °C until further analysis. Soil properties and vegetation characteristics of each forest area were measured and described previously (Köster et al., 2017; Zhou et al., 2019). Environmental factors were composed of abiotic variables (soil temperature, moisture, pH, active layer thickness and soil-available C, N and P) and biotic variables (fungal-to-bacterial ratio, microbial C and P, ground vegetation biomass and foliage biomass) (Tables 1 and A.1).

Table 1
Soil properties across four study areas. Values presented here are the means and standard errors (n = 9). Superscript letters represent the significant differences between four forest areas of each layer, with a significant level of 0.05.

Area	Depth cm	Active layer thickness cm	Organic layer thickness cm	pH	Soil available C (mg g^{-1})	Soil available N (mg g^{-1})	Soil type
$Fire_3$	5	101 ± 9[a]	5.34 ± 1.2[a]	4.54 ± 0.07[a]	1.95 ± 0.50[a]	0.42 ± 0.12[a]	Organic
	10			4.70 ± 0.06[a]	0.30 ± 0.04[a]	0.04 ± 0.01[a]	Mineral
	30			5.15 ± 0.14[a]	0.51 ± 0.12[a]	0.05 ± 0.01[a]	Mineral
$Fire_{25}$	5	88 ± 10[ab]	10.20 ± 2.5[b]	4.81 ± 0.15[a]	1.58 ± 0.32[a]	0.29 ± 0.09[a]	Organic
	10			5.16 ± 0.14[a]	1.03 ± 0.42[b]	0.19 ± 0.12[a]	Organic
	30			5.42 ± 0.11[a]	0.30 ± 0.05[a]	0.05 ± 0.01[a]	Mineral
$Fire_{46}$	5	49 ± 5[b]	14.11 ± 2.1[c]	6.64 ± 0.21[b]	5.51 ± 1.40[b]	0.26 ± 0.06[a]	Organic
	10			6.56 ± 0.14[b]	0.73 ± 0.12[b]	0.15 ± 0.06[a]	Organic
	30			7.02 ± 0.12[b]	0.43 ± 0.02[a]	0.02 ± 0.00[a]	Mineral
Control	5	28 ± 2[c]	16.00 ± 1.4[c]	4.77 ± 0.32[b]	9.73 ± 3.10[c]	0.48 ± 0.14[a]	Organic
	10			5.00 ± 0.22[b]	3.62 ± 1.07[c]	0.27 ± 0.07[b]	Organic
	30			5.68 ± 0.29[b]	0.96 ± 0.19[b]	0.10 ± 0.01[a]	Mineral

2.2. DNA extraction, amplification, and sequencing

DNA was extracted from a 0.25-g (fresh weight) soil sample using NucleoSpin Soil genomic DNA kit (Macherey-Nagel GmbH & Co) with the following modifications (Timonen et al., 2017). Briefly, soil samples were homogenized using FastPrep-24 Instrument in ceramic bead tubes at 5 m s^{-1} for 30 s. Instead of using the enhancer SX solution, optimal extraction conditions were built with Lysis Buffer SL1. Nucleic acids were eluted in 30 µl of elution buffer SE (5 mM Tris/HCl, pH 8.5). Extracted DNA was further purified using PowerClean Pro DNA Clean-Up Kit following the manufacturer's instructions (MoBio Laboratories). The DNA concentrations were quantified using the Qubit 2.0 Fluorometer (Thermo Fisher Scientific) and were diluted to 10 ng μl^{-1} with nuclease-free water.

PCR amplification of the bacterial V3–V4 region 16S rDNA and Illumina MiSeq sequencing were performed at the Institute of Biotechnology, University of Helsinki. PCR amplification was done in two steps. The first step used extracted DNA as templates and the bacteria-specific primers f341 and r785 (Klindworth et al., 2013) containing partial TruSeq adapter sequences at their 5′ end (ATC TAC ACT CTT TCC CTA CAC GAC GCT CTT CCG ATC T and GTG ACT GGA GTT CAG ACG TGT GCT CTT CCG ATC T, respectively). The second step used DNA produced during the first step as the templates, with the full-length TruSeq P5 and index containing P7 adapters as the primers. The final PCR amplicons were purified and sequenced using the pair-ended (PE-300) Illumina MiSeq Platform.

2.3. Data processing

The raw 16S rDNA reads were pre-processed at the Institute of Biotechnology, and the quality of the reads was checked using the FastQC software (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Adapter and barcode sequences were cut using the Cutadapt software (Martin, 2011).

The sequence data were filtered, de-noised and clustered into operational taxonomic units (OTUs) using mothur [version 1.39 (Schloss et al., 2009)] following the standard operating procedure (Kozich et al., 2013, accessed 15 January 2017). Briefly, reads that shorter than 200 bp and longer than 600 bp, containing ambiguous base call and homopolymers were removed. Furthermore, pre.cluster command were used to pre-cluster sequences with < 6 base differences and *chimera.vsearch* command were used to remove chimera (PCR error). To classify (*classify.seq* command) those sequences to taxonomic groups, the SILVA database [release 132; (Quast et al., 2013; Yilmaz et al., 2014)] with confidence threshold of 80% was used. Then, the taxonomic groups were assigned into OTUs with 97% similarities using *cluster.split* command in mothur with clustering method set to "average" (Wang et al., 2007). Global singletons (OTUs that contain only one sequence across all samples) were removed from the downstream analysis. The sequence data were clustered to 26,160 OTUs. To avoid taxa that seldom appeared among samples, OTUs that had < 5 reads globally in the dataset were removed (Callahan et al., 2016).

2.4. GeoChip 5.0K microarray

For GeoChip 5.0K analysis, 24 DNA samples from two soil layers (5 cm and 30 cm) with three replicates in each area were implemented. Replicates were obtained from the pooled-genomic DNA sample of the three soil plots in each forest stand. The genomic DNA was mixed with random primers and then labelled with 15-µl labelling master mix. The labelled genomic DNA was further purified, hybridised and scanned in the GeoChip 5.0K microarray as described in Van Nostrand et al. (2016). As part of Glomics Inc.'s customer service, the fluorescence intensity was pre-processed by removing outliers and data with low signal intensities according to the signal-to-noise ratio. High-quality signal intensities obtained from Glomics Inc. were normalised by dividing each gene frequency by the mean of the total frequency that multiplied with a constant as described previously (Van Nostrand et al., 2016).

2.5. Statistical analysis

All analyses were conducted using the R programming environment, version 3.5.2 (R Core Team, 2018). OTU counts were first normalised using the library sizes, and then pre-processed using *phyloseq* package (McMurdie and Holmes, 2013). Prior to statistical analysis, spatial area effects of bacterial community data (OTU level) between three burned areas ($Fire_3$, $Fire_{25}$ and $Fire_{46}$) and adjacent control forest stands were tested using the permutational multivariate analysis of variance (PERMANOVA, calculated using Bray-Curtis distances, 999 permutations). Area effects between three burned areas can be negligible since the difference between each burned area and the adjacent control forest stands (PERMANOVA, $F > 7, p < 0.001$) were larger than the difference among the three control stands ($F < 3, p > 0.01$). In addition, apart from soils at the 10-cm layer in $Fire_3$ were in mineral layers, those in the remaining areas were in organic layers. Soil types at 5 and 30 cm were the same across the four forest areas (Table 1). Soil types will be considered and discussed when comparing soil microbial properties at the 10-cm layer between forest areas.

We calculated bacterial diversity (Shannon's H and Fisher's α) and richness metrics (Chao1 index) using *diversity* function in *vegan* package (Oksanen et al., 2018) and analysed the statistical significance of these indices across the four forest areas using the one-way analysis of variance (ANOVA). All statistical significance analyses in this study were tested separately within each soil layer. Overall differences in the bacterial community composition at OTU level were tested through

PERMANOVA (calculated using Bray-Curtis distances, 999 permutations) using *adonis* function in *vegan* package. The differential gene expression analysis (DEA) based on a negative binomial distribution was applied to test the degree of taxonomic difference at phylum and class levels between burned forests and the control using *DESeq2* package (Love et al., 2014), applying a statistical significance level of $p_{adj} < 0.05$ and log2-fold change ≥ 1. Before multivariate analysis, the relative abundances were log-transformed to reduce the heteroscedasticity of the data. Canonical correspondence analysis (CCA) was performed to test the major gradient of the environmental variables (all variables are listed in Tables 1 and A.1) on explaining the changes in microbial community compositions among different forest areas. The contribution of each explanatory variable was determined using ANOVA-like permutation tests, implemented with *anova.cca* function. Since the environmental variables constituted two groups—that are abiotic variables and biotic variables—the contribution of each group on reshaping bacterial communities was determined using variance partitioning analysis (*varpart* function in *vegan* package).

GeoChip 5.0K contains 167,044 pre-determined probes to detect bacterial functional genes. Specifically, target genes involved in current study can be divided into six gene categories (i.e., C, N and P cycles, stress response, organic remediation and metal homeostasis), and 50 gene subcategories. Gene signal intensities were first aggregated by gene families (i.e., genes coding for protein with the same function) and were subsequently divided by the total gene frequencies of each gene category. The differences in overall gene pools (at the unique gene level) between different forest areas and soil profiles were analysed using PERMANOVA with Bray-Curtis dissimilarity indices. Statistical significances of the gene frequencies (at gene subcategory level) between the four forest areas were determined using the non-parametric Kruskal-Wallis test with *dunn.test* function in *dunn.test* package (Dinno, 2017). Prior to multivariate analysis, gene frequency data were transformed using Hellinger distances—a square-root conversion of the relative frequencies divided by the total frequency of all samples in each gene. We implemented CCA to determine the contribution of community assemblages and environmental variables on functional gene pools. The significance of the constraint variables was assessed using ANOVA-like permutation tests with *anova.cca* function. Variance partitioning analysis linking bacterial communities, biotic variables and abiotic variables to functional gene pools was also conducted (Oksanen et al., 2018). Prior to the variance partitioning analysis, bacterial community matrix (at phylum level) was converted to four principle components using principal component analysis (PCA) to prevent collinearity among taxa. Diagrams were drawn using *ggplot2* (Wickham, 2016) and *ComplexHeatmap* packages (Gu et al., 2016).

2.6. Data accessibility

The Illumina MiSeq sequencing data are available in the NCBI (National Centre for Biotechnology Information) database under Bioproject number PRJNA514982.

3. Results

3.1. Environmental factors in the study areas

Soil temperature (deep soil layers only), moisture, soil available C and N and ground vegetation biomass showed significant differences between young ($Fire_3$ and $Fire_{25}$) and old ($Fire_{46}$ and the control) forest areas (Tables 1 and A.1). Soil available C at 5-cm layer was substantially increased in $Fire_{46}$ and the control soils in particular. Soil pH in $Fire_{46}$ was the highest among the four forest areas, which did not show a clear trend with ages following a fire (Table 1).

3.2. Bacterial community composition and diversity

After quality control and noise removal that described in the method, 6,438,536 high-quality reads across all 107 samples remained, ranging from 36,900 to 76,474 reads per sample with an average of 60,173 (± 7679) reads. All reads were classified to the bacterial domain of 26,160 OTUs per sample and further de-noised to 11,006 OTUs per sample by removing OTUs with < 5 reads globally in the dataset (Callahan et al., 2016). These 11,006 OTUs covered 43 phyla, 160 classes, 262 orders and 489 genera. The most abundant phylum across all samples was Proteobacteria (31.2% of the total sequence count, 29.1% of OTUs), followed by Actinobacteria (19% of the total sequence count, 9.5% of OTUs), Acidobacteria (18.6% of the total sequence count, 8.5% of OTUs), Chloroflexi (11% of the total sequence count, 5% of OTUs) and Planctomycetes (3.6% of the total sequence count, 11% of OTUs). Less abundant phyla, including Verrucomicrobia, Bacteroidetes, Gemmatimonadetes and Firmicutes, represented 15.4% of the total sequence count and 17% of OTUs. The remaining phyla represented 3.6% of all sequences and 19.9% of OTUs.

In a comparison of alpha diversities of bacteria between the four fire areas at each soil layer, no significant difference was found between $Fire_3$, $Fire_{25}$ and the control, but significant differences were obvious between $Fire_{46}$ and the remaining areas (ANOVA, $p < 0.03$, Table A.2): bacterial richness, diversity and evenness of surface soils in $Fire_{46}$ were highest across all study areas.

At the 5-cm layer, relative abundances of Chloroflexi and Proteobacteria in $Fire_3$ were significantly differed with the control among the ten most abundant phyla. Chloroflexi was more abundant in $Fire_3$ than in the control (log_2-fold change = 2.2, $p < 0.05$; Table A.3), while the abundance of Proteobacteria showed the opposite (log_2-fold change = −0.5, $p < 0.05$; Table A.3). Increase in abundances of Chloroflexi mainly relied on the increase in class Ktedonobacteria (log_2-fold change = 4.3, $p < 0.001$; Table 2), as the remaining classes of Chloroflexi were either less abundant (Anaerolineae and Chloroflexia) or remain unchanged (Thermomicrobia) compared with the control (log_2-fold change < −4.0, $p < 0.001$; Table 2). At class level, relative abundances of Blastocatellia (Actinobacteria), Cytophagia (Bacteroidetes), Opitutae and Verrucomicrobiae (Verrucomicrobia) significantly changed 3-year postfire comparing with the control (Table 2). Although no significant differences in bacterial communities at phylum and class levels were found between $Fire_{25}$ and the control, taxa differences between $Fire_{46}$ and the control were evident (Table 2). Results showed that $Fire_{46}$ harboured higher abundance of Acidimicrobiia and Holophagae (Acidobacteria), Thermoleophilia and Blastocatellia (Actinobacteria), Thermomicrobia (Chloroflexi), Bacilli (Firmicutes) and Gemmatimonadetes, but lower abundance of Solibacteres (Acidobacteria), Clostridia (Firmicutes), Phycisphaerae (Planctomycetes) and Opitutae (Verrucomicrobia).

At the 10-cm layer, relative abundances of Acidobacteria, Chloroflexia, Alpha- and Gammaproteobacteria were significantly lower in $Fire_3$ but Solibacteres and Ktedonobacteria were higher than those in the control (Tables 2 and A.3). These differences were probably driven by soil types (organic or mineral), soil temperature or both. Similarly, relative abundances of Chloroflexia, Alpha- and Gammaproteobacteria were lower in $Fire_{25}$ compared with the control (Table 2). Most bacterial classes in $Fire_{46}$ retained similar uniqueness as it at 5 cm layers (Table 2).

Relative abundances of bacterial phyla at the 30-cm soil layers showed no significant difference between recently burned forest soils ($Fire_3$ and $Fire_{25}$) and the control (Tables 2 and A.3). On the contrary, many bacterial classes in $Fire_{46}$ significantly differed in their abundances with the control, such as Acidobacteria, Holophagae (Acidobacteria), Blastocatellia (Actinobacteria) Chloroflexia and Ktedonobacteria (Chloroflexi) and so on (Table 2).

Across the soil profile, Acidobacteria, Actinobacteria and Proteobacteria were predominant in the organic soils but played a less

Table 2
Relative abundance (%) of bacterial classes (average abundance; $n = 9$) across four forest areas of three soil layers. Phyla and classes that significantly more abundant (↑) or less abundant (↓) between burned forests ($Fire_3$, $Fire_{25}$ and $Fire_{46}$, respectively) and the control are labelled in bold.

		5 cm				10 cm				30 cm			
Phylum	Class	$Fire_3$	$Fire_{25}$	$Fire_{46}$	Control	$Fire_3$	$Fire_{25}$	$Fire_{46}$	Control	$Fire_3$	$Fire_{25}$	$Fire_{46}$	Control
Acidobacteria	**Acidobacteria**	11.78	12.45	**1.64 ↓**	10.15	**7.29 ↓**	8.19	0.94	12.56	3.8	3.32	**0.25↓**	3.69
	Acidimicrobiia	4.56	3.78	4.23	2.43	4.05	3.79	4.93	4.68	3.08	2.85	3.93	3.9
	Holophagae	0.22	0.22	**0.6 ↓**	0.13	1.23	1.09	1.93	0.56	1.96	1.51	**3.34↑**	1.89
	Solibacteres	4.07	5.14	**1.35 ↓**	2.31	**4.8 ↑**	5.37	1.62	2.55	3.25	3.36	1.11	2.84
	Actinobacteria	8.52	6.84	10.61	7.38	5.91	6.29	5.11	7.41	5.77	3.67	3.37	5.5
Actinobacteria	**Blastocatellia**	**0.01 ↓**	0.09	**1.44 ↑**	0.24	0.22	0.29	**2.17 ↑**	0.64	0.5	0.54	**3.2↑**	1.13
	Coriobacteriia	0	0	0	0	0.01	0.03	0.01	0	0.17	0.03	0.04	0.14
	Thermoleophilia	3.34	2.44	**7.3 ↑**	2.22	4.2	3.84	**12.12↑**	3.73	4.6	4.02	15.75	6.64
Bacteroidetes	**Sphingobacteriia**	2.67	2.86	3.36	2.86	**2.2 ↓**	2.38	2.3	3.38	1.94	1.91	1.45	1.98
	Cytophagia	**0.06 ↓**	0.14	0.68	0.22	0.07	0.19	0.19	0.29	0.04	0.09	0.11	0.12
	Bacteroidia	0	0	0	0	0.01	0.01	0	0.01	0.6	0.04	**0↓**	0.19
Chloroflexi ↑	**Ktedonobacteria**	**2.2 ↑**	0.69	0.07	0.11	**3.28 ↑**	2.81	0.1	0.63	4.83	5.12	**0.12↓**	1.92
	Chloroflexia	**0 ↓**	0.01	0.26	0.07	**0 ↓**	**0.01 ↓**	0.47	0.12	0.02	0.09	**0.48↑**	0.09
	Anaerolineae	**0.01 ↓**	0.04	0.21	0.11	0.08	0.07	0.23	0.13	0.2	0.22	0.24	0.21
	Thermomicrobia	0	0.01	**0.1 ↑**	0.02	0.01	0.02	**0.1 ↑**	0.03	0.03	0.05	**0.15 ↑**	0.07
Firmicutes	Clostridia	0.84	1.48	**0.33 ↓**	0.71	0.98	1.17	**0.22 ↓**	0.65	1.07	0.5	0.27	0.87
	Bacilli	0.03	0.03	**0.85 ↑**	0.05	0.1	0.03	**0.62 ↑**	0.06	0.1	0.07	**1.44 ↑**	0.19
Gemmatimonadetes	Gemmatimonadetes	0.46	0.37	**2.79 ↑**	0.24	2.08	1.93	**4.32 ↑**	1.29	3.09	3.72	**6.59 ↑**	4.21
Parcubacteria	Nomurabacteria	0.05	0.08	0.17	0.04	0.08	0.07	0.16	0.13	0.46	0.15	0.2	0.64
	Moranbacteria	0	0	0.03	0	0.01	0	0.08	0.01	0.07	0.02	0.09	0.28
	Azambacteria	0.05	0.04	0.04	0.02	0.05	0.03	0.04	0.04	0.1	0.06	0.03	0.16
Planctomycetes	Planctomycetacia	3.35	3.02	2.13	2.1	2.5	**1.93 ↓**	**1.25 ↓**	2.38	1.37	1.17	0.61	1.16
	Phycisphaerae	1.83	1.77	**0.83 ↓**	1.11	1.33	1.33	0.74	1.16	0.75	0.83	0.44	0.62
Proteobacteria ↓	**Alphaproteobacteria**	**17.54↓**	20.17	25.29	18.64	**17.57↓**	**16.02↓**	**14.94↓**	18.46	10.63	9.82	6.68	11.84
	Betaproteobacteria	**1.92↓**	3.33	5.05	3.69	4.93	4.04	7.69	4.86	6.86	6.33	9.09	9.36
	Gammaproteobacteria	5.28	6.67	4.34	5.51	**3.08 ↓**	**3.79 ↓**	1.4	6.94	1.36	1.22	0.45	1.68
	Deltaproteobacteria	3.99	3.33	2.52	2.49	2.88	2.65	**3.02 ↑**	2.98	2.02	2.45	**2.88↑**	2.27
Saccharibacteria	Saccharibacteria_class	0.47	0.49	0.83	0.48	0.38	0.59	0.35	0.75	0.51	0.4	0.2	0.71
Verrucomicrobia	Opitutae	1.57	0.93	0.16	0.59	0.65	0.67	**0.12↓**	0.81	0.48	0.31	0.14	0.39
	Spartobacteria	1.84	1.89	2.88	1.39	4.3	2.85	**3.22↑**	2.18	3.36	3.02	2.33	2.33
	Verrucomicrobiae	0.02	0.02	0.07	0.03	0.03	0.02	0.06	0.04	0.03	0.03	0.06	0.06
Others		23.32	21.67	19.84	34.66	25.69	28.5	29.55	20.54	36.95	43.08	34.96	32.92

The classes that significant differed between $Fire_3$ and the control are shown in bold. The intensity of colour represents the relative abundance of classes in soils of each area. Results of significance tests were listed in Table A.3.

important role in the mineral soils, except for $Fire_{46}$ where Acidobacteria was less but Actinobacteria and Proteobacteria were relatively more abundant.

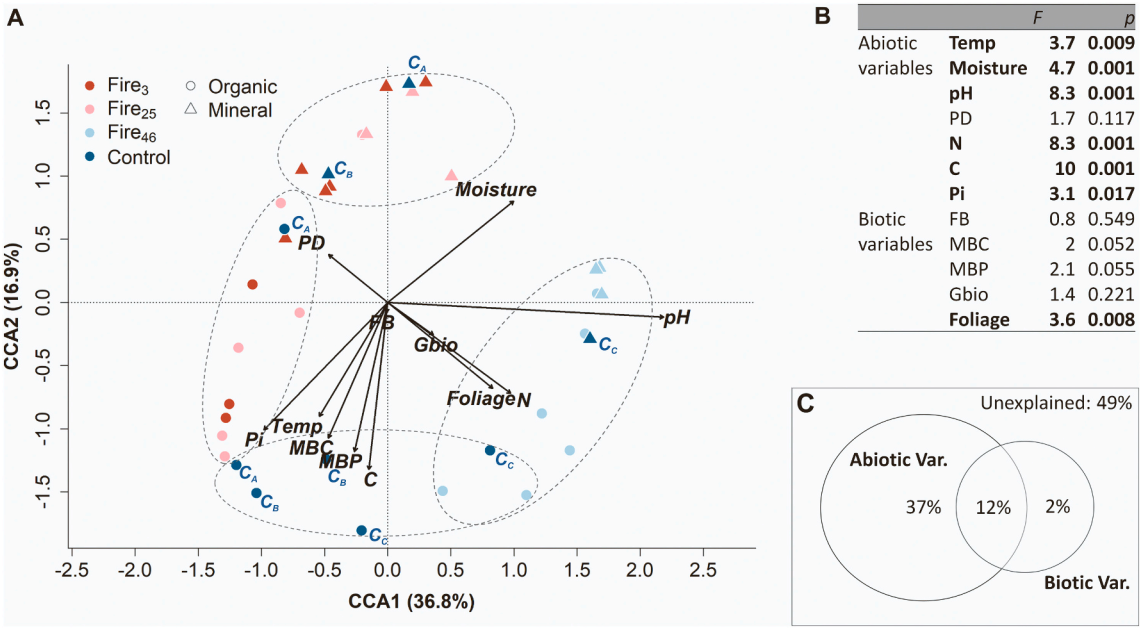
At OTU level, we tested significant differences in bacterial communities among the four forest areas (PERMANOVA; $r^2 = 0.21$, $p < 0.001$) and soil layers ($r^2 = 0.15$, $p < 0.001$; Table 3). Pairwise comparison demonstrated that bacterial communities in $Fire_3$ significantly differed from $Fire_{46}$ and the control ($F > 3.92$, $p < 0.001$), but not from $Fire_{25}$ ($F = 1.34$, $p > 0.1$; Table 3). Differences in bacterial communities between $Fire_{46}$ and the remaining areas were rather considerable ($F > 12$, $p < 0.001$; Table 3), which were even larger than differences between $Fire_3$ and the control ($F = 3.92$, $p < 0.001$). In addition, bacterial community compositions across the soil profile were significantly different ($F > 5.9$, $p < 0.001$, Table 3).

The ordination diagram of CCA showed a general pattern of bacterial community composition in soils across four areas that distributed along the gradient of environmental variables (Fig. 1A). Overall, the first two axes of the CCA explained 53.7% of the variation in community composition (Fig. 1A). Projecting the sample points and environmental factors on CCA1 axis, it showed that bacterial communities in $Fire_{46}$ were positively correlated with soil pH, soil N content, moisture, and tree foliage biomass (ANOVA-like permutation test; $F > 3.8$, $p < 0.01$); other areas, except for mineral soils, were positively correlated with inorganic P ($F = 3.1$, $p < 0.05$) but negatively with soil moisture ($F = 4.7$, $p < 0.001$; Fig. 1A and B). The projection of sample points on CCA2 showed that bacterial communities in the control were positively correlated with soil available C contents, inorganic P and temperature ($F > 3.1$, $p < 0.05$; Fig. 1A and B).

Variance partitioning analysis showed that abiotic variables

Table 3
Permutational analysis of variance (PERMANOVA) shows the difference between burned forests and the control based on the Bray-Curtis dissimilarity indices performed on community data at the OTU level. *p*-values at a significant level of < 0.05 were shown in bold.

Tested variables	df	MS	Pseudo F	r^2	*p*
Total areas					
Stand age	3	2.02	10.52	0.21	**0.001**
Depth	2	2.23	11.62	0.15	**0.001**
Residuals	99	0.19			
$Fire_3$ vs. Control	1	0.82	3.92	0.06	**0.001**
Depths	2	1.38	6.60	0.20	**0.001**
Residuals	48	0.21			
$Fire_{25}$ vs. Control	1	0.66	2.98	0.05	**0.011**
Depths	2	1.36	6.12	0.20	**0.001**
Residuals	47	0.22			
$Fire_{46}$ vs. Control	1	2.33	11.88	0.17	**0.001**
Depths	2	1.16	5.88	0.16	**0.001**
Residuals	48	0.20			
$Fire_3$ vs. $Fire_{25}$	1	0.24	1.34	0.02	0.17
Depths	2	1.56	8.87	0.26	**0.001**
Residuals	49	0.17			
$Fire_3$ vs. $Fire_{46}$	1	4.17	26.25	0.29	**0.001**
Depths	2	1.17	7.37	0.16	**0.001**
Residuals	50	0.16			
$Fire_{25}$ vs. $Fire_{46}$	1	3.79	22.08	0.26	**0.001**
Depths	2	1.12	6.51	0.15	**0.001**
Residuals	49	0.17			



		F	p
Abiotic variables	**Temp**	**3.7**	**0.009**
	Moisture	**4.7**	**0.001**
	pH	**8.3**	**0.001**
	PD	1.7	0.117
	N	**8.3**	**0.001**
	C	**10**	**0.001**
	Pi	**3.1**	**0.017**
Biotic variables	FB	0.8	0.549
	MBC	2	0.052
	MBP	2.1	0.055
	Gbio	1.4	0.221
	Foliage	**3.6**	**0.008**

Fig. 1. Results of the canonical correspondence analysis (CCA) in the Illumina MiSeq sequencing data. (A) CCA based on the Bray-Curtis distances shows the clustered bacterial communities at the OTU level. Each point in red represents samples from $Fire_3$, pink represents $Fire_{25}$, light blue represents $Fire_{46}$ and blue represents the control. The circular points represent samples of organic soils, and the triangular points represent samples from mineral soils. Samples from different control stands were labelled according to their locations on the map (see Fig. A.1). (B) Contributions of the environmental variables to different bacterial community were tested using the ANOVA-like permutation test. Environmental variables were divided into abiotic variables and biotic variables. Explanatory factors that significantly correlated ($p < 0.05$) with variations in the bacterial community structure are highlighted in bold. (C) The contributions of the abiotic variables and biotic variables to the variation in bacterial community were tested using variance partitioning analysis and are represented as a Venn diagram.
†Abbreviations: Temp: soil temperature; PD: permafrost depth; FB: fungal-to-bacterial ratio; MBC and MBP are microbial biomass C and P contents (mg g^{-1}); Gbio: ground vegetation biomass; Foliage: tree foliage biomass (kg m^{-2}). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

explained around 37% of the observed variation in the bacterial community compositions, whereas biotic variables only explained 2% of the variance. The interaction between biotic and abiotic variables explained another 12% of the variance (Fig. 1C, variance partitioning).

3.3. Bacterial functional gene pool and diversity

A total of 853 gene families across all samples were detected using GeoChip 5.0K microarray. The detected bacterial gene families in this study were categorised based on six primary metabolic processes: the C, N and phosphorus (P) cycles; stress response; organic remediation; and metal homeostasis. We found no significant differences in the diversity, richness and evenness of bacterial functional gene pools between the four areas.

To understand how wildfire affects soil bacterial potential functions, we combined the detected intensities of genes coding for the same metabolic function. In the surface layer, genes coding for carbon degradation, nitrogen fixation and anaerobic ammonium oxidation (anammox) pathways were found more frequent in $Fire_3$ than in the control ($p < 0.05$), along with genes coding for metal (aluminium, arsenic, iron, calcium and mercury) homeostasis ($p < 0.05$; Fig. 2). Surprisingly, other genes, especially those involved in C and N cycles, remained unchanged 3 years postfire compare with the control. Significant differences between $Fire_{46}$ and the control were also detected, revealing higher frequencies of nitrogen fixation, cold shock, arsenic and cobalt homeostasis genes ($p < 0.05$, Fig. 2). When compare gene frequencies of the deep soil layer among the four forest areas, although it showed an increasing trend postfire (Fig. 2), the difference was nevertheless insignificant.

CCA revealed that functional gene pools in surface layer differed among the forest areas, while the differences were not consistent in deep soil layers (Fig. 3A). Variation in functional gene composition on first two CCA axes was 29% (Fig. 3A). Bacterial functional gene pools in burned forest soils ($Fire_3$ and $Fire_{25}$) were distinct from those in old-growth forests ($Fire_{46}$ and the control) (PERMANOVA, $F > 2.1$, $p < 0.05$). Functional gene pools in surface soils of $Fire_3$ were also distinct from that of $Fire_{25}$ ($F = 3.5$, $p < 0.01$). In the deep soil layer, functional genes of all areas grouped closer compared to those in the surface layer. Projecting sample points and the explaining factors on CCA1, it showed soil inorganic P (ANOVA-like permutation test; $F > 2.8$, $p < 0.001$) was positively correlated with functional gene pools in $Fire_{25}$ but negatively correlated with old forest areas ($Fire_{46}$ and the control) (Fig. 3A and B). In addition, soil moisture ($F = 2.5$, $p < 0.01$) and foliage biomass ($F = 1.8$, $p < 0.05$) positively correlated with that in $Fire_{46}$. When projecting samples on CCA2, soil temperature ($F = 3.0$, $p < 0.001$), permafrost depth (active layer thickness; $F = 1.7$, $p < 0.05$) and soil available C contents ($F = 2.4$, $p < 0.01$) were positively correlated with functional gene pools in $Fire_3$, while with ground vegetation biomass ($F = 2.5$, $p < 0.01$) was negatively correlated (Fig. 3A and B).

Variance partitioning analysis indicated that soil bacterial communities explained 20% of functional gene variations, while abiotic and biotic variables explained 24% and 15%, respectively. Shared effects of the variable groups ranged from 5% to 8% (Fig. 3C). Unexplained variances accounted for 23%.

4. Discussion

In the boreal forest, wildfire typically alters the quantity and quality of soil organic matters and deepens active layer thickness (Certini, 2005; Michaelides et al., 2019). As such, soil bacterial communities in burned forests were taxonomically and functionally different from those in unburned forests. In particular, relative abundances of Chloroflexi increased but Betaproteobacteria decreased shortly postfire. We

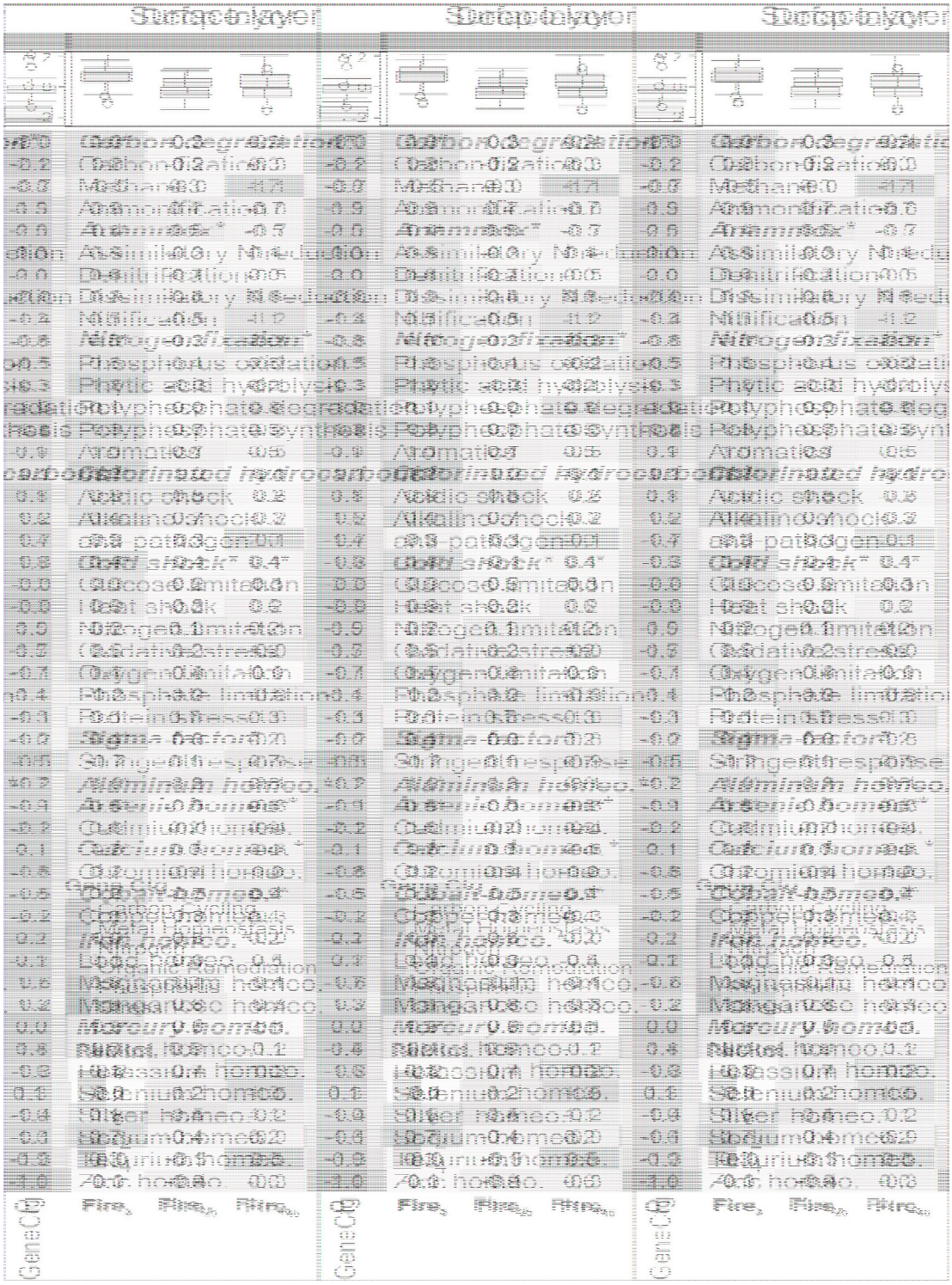


Fig. 2. Heatmap of the gene categories (subcategory 1 in GeoChip 5.0) showing the changes in relative intensities (scale gene frequencies to the zero mean and unit variance) across the four forest areas. Significant differences in the functional gene frequencies between fire-affected forests and the control are presented in bold with an asterisk for those with a significance level of < 0.05. The boxplots above the heatmap represent the total relative frequency of genes in each treatment.

also found that functional genes, such as genes coding for C degradation and ammonium oxidation, were more frequent in recently burned soils than in unburned soils. Despite microbial functions are performed by individual microorganisms and many researchers expect to predict ecosystem functions by assessing microbial community shifts (Pérez-Valera et al., 2019; Salles et al., 2009; Tan et al., 2012), bacterial potential functions found in this study correlated more strongly with the changing environment than the changing community composition. This underpins the evidence regarding functional redundancy in bacterial communities as indicated in earlier studies (Louca et al., 2018). Considering warming effects on permafrost, previous study demonstrated that the C and N cycling genes shift rapidly following permafrost thaw (Mackelprang et al., 2011). Although we found an increasing trend of few functional genes in the near-postfire permafrost layer, the differences were too small to be significant.

Relative abundances of Proteobacteria decreased 3 years postfire,

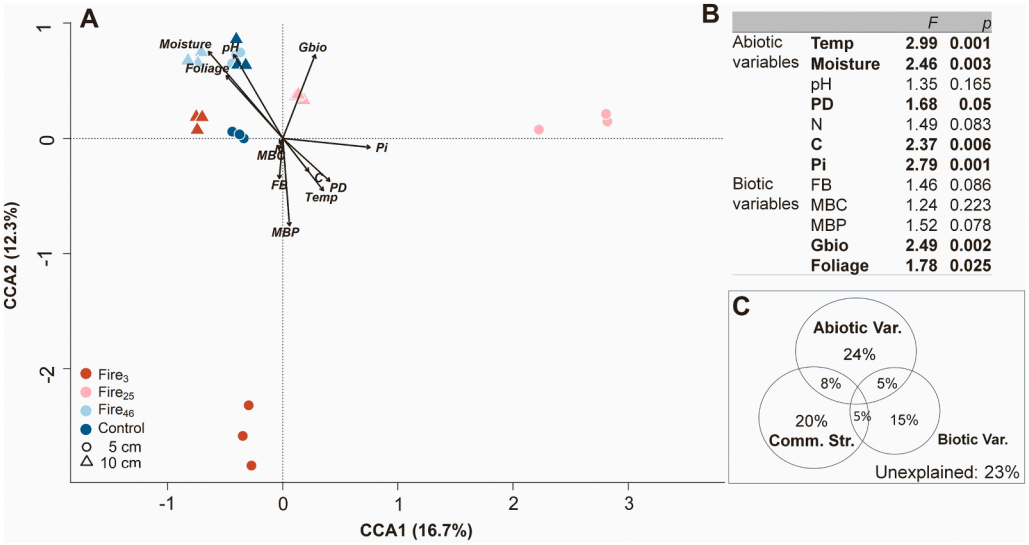


Fig. 3. Results of the canonical correspondence analysis (CCA). (A) The functional gene pools clustered using the Bray-Curtis distance matrix. Each point in red represents samples from $Fire_3$, pink represents $Fire_{25}$, light blue represents $Fire_{46}$ and blue represents the control. Circular points represent samples of organic soils, and triangular points represent samples from mineral soils. (B) Contributions from environmental factors calculated using the ANOVA-like permutation test. Environmental variables were divided into abiotic variables and biotic variables. Explanatory factors that significantly correlated ($p < 0.05$) with the functional gene pools are highlighted in bold. (C) The contributions of abiotic variables, biotic variables and the bacterial community structure (at the phylum level) to the observed functional gene pool are shown in the Venn diagram.

†Abbreviations: Temp: soil temperature; PD: permafrost depth; FB: fungal-to-bacterial ratio; MBC and MBP are microbial biomass C and P contents (mg g^{-1}); Gbio: ground vegetation biomass; Foliage: tree foliage biomass (kg m^{-2}). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

while that of Chloroflexi increased (Table 2). These shifts in bacterial taxa assemblages did not last long, given that in forests 25 years postfire the difference between burned forests and the control was no longer significant. Boreal forests are typically dominated by Proteobacteria, Actinobacteria, Acidobacteria, Bacteroidetes and Chloroflexi (Steven et al., 2007; Taş et al., 2014; Wilhelm et al., 2011). Previous studies found that a < 1 year postfire area appeared to have positive effects on soil Betaproteobacteria, as well as Actinobacteria, Proteobacteria and Firmicutes (Prendergast-Miller et al., 2017; Smith et al., 2008; Xiang et al., 2014). On the contrary, our results revealed a negative effect of fire on Betaproteobacteria 3-year postfire. In seven-year (Taş et al., 2014) and 11-year postfire soils (Xiang et al., 2014), the abundance of all members in Proteobacteria did not differ from the unburned forest soils. These contradictions may result from different postfire forests one used. The more recently burned forests, its soils are more likely abundant with nutrients (Certini, 2005), which may affect microbial community compositions. Additionally, the abundance of Chloroflexi was increased in burned soils. As Chloroflexi has rarely been studied in natural environments, its role in decomposing organic C remains unknown. Although Taş et al. (2014) found Chloroflexi is negatively affected by fire (Taş et al., 2014), a fire study in a coal mine found that Chloroflexi was more abundant in fire-affected soils (Lee et al., 2016). Yet, when we studied the genera of Chloroflexi, we found only a significant increase in Ktedonobacteria in $Fire_3$ compared to the control, while Anaerolineae and Chloroflexia decreased (Table 2). The increased in Ktedonobacteria was likely related to its ability to form spores (Cavaletti et al., 2006; Yabe et al., 2010), which were prominent in harsh environments such as in volcanic deposits or in soils with extremely low C contents (Lynch et al., 2012; Weber and King, 2010).

Unexpectedly, we found several distinctive bacterial taxa in $Fire_{46}$ compared to the remaining forest areas (Table 2). We provided further evidence indicating that high soil pH dramatically reshapes bacterial communities. Although some studies found fire alters soil pH (Arocena and Opio, 2003; Ulery et al., 1993), we found that the change in soil pH has no pattern with time following a fire (Table 1). Thus, soil pH more likely depends on the mineral decomposition of the soil's parent material in current study. Here, we found soil pH had a substantial effect on the composition of bacterial communities in $Fire_{46}$ by increasing the abundance of Actinobacteria, but decreasing the abundance of Acidobacteria (Fig. 1 and Table 2). This finding is consistent with previous studies in which a higher soil pH typically caused a higher abundance of Actinobacteria and a lower abundance of Acidobacteria (Griffiths et al., 2011; Lauber et al., 2009).

Wildfire altered many potential functions of the bacterial community, such as genes involved in C and N cycles, the metal homeostasis and the organic remediation (Fig. 2). It is worth noting that the gene pools found in our study were DNA-based, merely reflecting potential functions of bacterial communities instead of the real metabolic activity. Nevertheless, we still found a clear pattern of functional gene pools among the four forest areas. Genes coding for C degradation were more frequent in surface soils in $Fire_3$ than that in the control. This agrees with previous studies which found that fire can increase heterotrophic respiration (O'Neill et al., 2003; Richter et al., 2000), although an unchanged respiration rate has also been observed (Irvine et al., 2007). In our previous study, CO_2 fluxes measured from the soil surface decreased after fire (Köster et al., 2017). However, this C release also contains respiration from plant roots, which decreased after fire due to the vegetation removal. A similar result was also found in O'Neill et al. (2003), where the CO_2 fluxes from burned soils were highly correlated with re-growing vegetation.

Genes coding for N fixation and anammox processes were more frequent in recently burned soils (Fig. 2), although previous studies found the abundance of N-fixing species decreased in response to fire (Kennedy and Egger, 2010; Yeager et al., 2005). However, anammox is a process that transfers ammonium and nitrite to nitrogen gas (Kuenen, 2008), that its increase may offset the increasing N-fixation process. Nonetheless, neither of these processes produce nitrous oxide (N_2O). This is consistent with our previous gas-flux study showing that the change in N_2O emissions after fire was negligible (Köster et al., 2017). Similar findings were also reported in burned soils in permafrost regions (Taş et al., 2014; Yeager et al., 2005).

Although we expected a strong link between bacterial community composition and its potential functions, the bacterial community composition merely explained about 20% of the functional gene variation, much less than that explained by biotic and abiotic environment (about 39%; Fig. 3C). This again supported functional redundancy in bacterial communities (Fernandez-Gonzalez et al., 2016; Vanwonterghem et al., 2016).

Genes involved in metal homeostasis, organic remediation, phosphorus metabolism and stress responses were consistently elevated following a fire (Fig. 2). Although data on the dynamic of metal content postfire remain scarce, studies have found that fire increases manganese

contents (Gonzalez Parra et al., 1996), and incombustible metals like magnesium, iron, lead and cobalt contents (Certini, 2005). In this sense, individuals containing metal-resistant genes would be more likely to survive. Furthermore, both complete and incomplete combustion of SOM gathers toxic compounds. For example, polycyclic aromatic hydrocarbons appeared to increase postfire (Koh et al., 2004). This could explain the higher frequency of hydrocarbon decomposition-related genes postfire (Fig. 2).

In addition to fire-induced changes in the surface layer bacterial communities and their potential functional gene pools, we expected a similar effect on fire in the deep soil layer due to changes in soil temperature. Surprisingly, bacterial communities at 10- and 30-cm layers remained stable across all areas, even when comparing the phyla between melted and frozen near-surface permafrost layers (30-cm layer). This likely stems from bacterial communities in permafrost consisting of slow-growing clades that can persist under C and nutrient starvation. By contrast, with a sufficient supply of organic substrates, such as carbohydrates and amino acids, permafrost microbial community decomposes more substrates at 20 °C than at 1 °C (Ernakovich and Wallenstein, 2015). Similarly, an incubation study revealed that both the microbial community and the genes involved in C and N cycling respond rapidly to permafrost thaw, thereby increasing methane emissions (Mackelprang et al., 2011). However, the permafrost soils used in these studies are from the deep permafrost layer, which suffers from constant cold environment compare to near-permafrost layer that may still melt in hot summer. In addition, Mackelprang et al.'s (2011) study was situated in Hess Creek, Alaska, which features organic-enriched soil even in the permafrost layer with soil C contents four to ten times higher than those in our study in northern Canada, probably also causing the difference. Another possibility might be that the functional gene frequencies observed here (DNA-based functional gene) merely reflected the stored gene pool, which may be inactive in frozen permafrost although gene frequencies remained consistent with those in the thawed permafrost. Thus, we suggest that future studies should employ a combination of both DNA- and RNA-based approaches to explore the activated microbial functions.

To conclude, this study indicates that wildfire significantly reshaped bacterial community composition and its potential functions in the surface soil layer, but did not shift those in the deep soil layer. Although we failed to observe any significant differences between thawed and frozen near-surface permafrost, bacterial communities in frozen soils may be largely inactive given the frozen conditions. The differences in bacterial community composition and its functional potentials between burned and unburned forests were primarily determined by abiotic variables such as the soil temperature, moisture and available soil nutrients. A valid concern is that the functional gene pool determined in this study was DNA based, reflecting only those genes stored in soils. Therefore, the active functions of the microbial communities in our study areas are uncertain. Overall, our work highlights bacterial fitness over a fire-induced environmental gradient in a continuous permafrost region, improving our understanding of temporal shifts in bacterial community compositions and their abilities to control C and N cycles postfire.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.apsoil.2020.103713.

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